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Biological Effects of Continuous and Intermittent Dosing of Outdoor Experimental Streams with Chlorpyrifos

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16. ABSTRACT

Two outdoor experimental streams fed by water pumped from the Mississippi River were dosed with the organophosphorus pesticide chlorpyrifos (the active ingredient of Dursban® and Lorsban 4E® insecticides) for 100 days, and the responses of individual species and communities compared to those of a control stream. Chlorpyrifos was continuously metered into one stream whereas the other received 8 biweekly additions, each lasting 24 h, at 14 times the concentration in the continuously dosed stream. Therefore, nearly equal amounts of pesticide were introduced into each stream during the experiment. The biological study area of each stream was approximately 245 m long, contained a naturally colonizing plant and invertebrate assemblage, and was stocked with fathead minnows and bluegills. Measured system characteristics included macroinvertebrate drift and riffle benthos composition; fish survival, growth, reproduction, food habits, tissue residues, and AChE inhibition; and system functional process indicators (*P/R* ratios, biodegradation, nitrate and dissolved organic carbon [DOC] concentrations, and bacterial growth and heterotrophic activity).

The macroinvertebrate communities reacted similarly in the continuously and pulse-dosed streams. Species diversity decreased by equal amounts and was still decreasing at the end of the test. Crippling of fathead minnows and reversible acute toxicity symptoms (lethargy, tetany when startled) in bluegills were observed only in the pulse-dosed stream. Fish survived, reproduced, and grew equally well in all streams. There seemed to be good agreement between laboratory and field effect concentrations for fish and invertebrates. Functional process indices, with the possible exception of biodegradation, appeared unaffected and considerably less sensitive than other characteristics measured. The results of the project are discussed in relation to lab to field extrapolation and the need for further testing.

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ABSTRACT: Two outdoor experimental streams fed by water pumped from the Mississippi River were dosed with the organophosphorus pesticide chlorpyrifos (the active ingredient of Dursban® and Lorsban 4E® insecticides) for 100 days, and the responses of individual species and communities compared to those of a control stream. Chlorpyrifos was continuously metered into one stream whereas the other received 8 biweekly additions, each lasting 24 h, at 14 times the concentration in the continuously dosed stream. Therefore, nearly equal amounts of pesticide were introduced into each stream during the experiment. The biological study area of each stream was approximately 245 m long, contained a naturally colonizing plant and invertebrate assemblage, and was stocked with fathead minnows and bluegills. Measured system characteristics included macroinvertebrate drift and riffle benthos composition; fish survival, growth, reproduction, food

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habits, tissue residues, and AChE inhibition; and system functional process indicators (*P/R* ratios, biodegradation, nitrate and dissolved organic carbon [DOC] concentrations, and bacterial growth and heterotrophic activity).

The macroinvertebrate communities reacted similarly in the continuously and pulse-dosed streams. Species diversity decreased by equal amounts and was still decreasing at the end of the test. Crippling of fathead minnows and reversible acute toxicity symptoms (lethargy, tetany when startled) in bluegills were observed only in the pulse-dosed stream. Fish survived, reproduced, and grew equally well in all streams. There seemed to be good agreement between laboratory and field effect concentrations for fish and invertebrates. Functional process indices, with the possible exception of biodegradation, appeared unaffected and considerably less sensitive than other characteristics measured. The results of the project are discussed in relation to lab to field extrapolation and the need for further testing.

KEY WORDS: pesticides, toxicity, fishes, invertebrates, field tests, bioassay, acetylcholinesterase inhibition, Dursban®, chlorpyrifos, bioconcentration, field validation

Very little information is available that quantitatively relates the effects of a toxicant on individual organisms in the laboratory to its effects on populations and communities in the field at well characterized exposure concentrations. Acquisition of such data is important for determining the utility of various hazard evaluation procedures and was the major focus of this investigation. Extensive analyses of chlorpyrifos concentrations in aquatic ecosystem components were conducted to determine organism exposures and to evaluate the fate prediction capabilities of the Exposure Analysis Modeling System (EXAMS) model developed by the U.S. Environmental Protection Agency (EPA) Environmental Research Laboratory—Athens (reported separately by Erickson [*1*]).

Chlorpyrifos was selected as the toxicant because of interest in its ecological effects by the U.S. EPA Office of Pesticide Programs, and because of the relatively large body of published test data to which the stream study results could be compared. Chlorpyrifos is commonly applied to ponds as a mosquito larvicide, recommended control rates being 28- to 56-g active ingredient per hectare (equal to 9 to 18 μg/L in 152 mm [6 in.] of water).

A comprehensive literature review of chlorpyrifos toxicity has been prepared by Marshall and Roberts [*2*]. Data on the effects of chlorpyrifos on nontarget macroinvertebrates consist mostly of short-term static exposures. Sanders [*3*] determined the median lethal concentration for the amphipod *Gammarus lacustris* in four days to be 0.1 μg/L. Other freshwater insects and crustaceans are generally less sensitive acutely than amphipods [*4–7*] although Muirhead-Thompson [*8*] found the mayfly *Baetis* to be more sensitive when exposed for 1 h and observed for the following 24 h. Moshen and Mulla [*9*] obtained a 24-h LC_{50} value of 0.9 μg/L for *Baetis parvus*.

Laboratory studies that exposed fish to chlorpyrifos are more numerous and include longer term tests in flowing water systems. Centrarchids are the most sensitive group tested acutely [*10*], these fish being somewhat more tolerant than the most sensitive invertebrates. Chronic studies have all employed

fathead minnows [*11,12*], a species two orders of magnitude more resistant acutely than bluegills. Chronic values for fatheads are lower than bluegill acute values and comparable to those of the most sensitive invertebrates (0.1 to 0.2 μg/L).

Several pool or pond studies have been conducted to determine aquatic community responses to standard or slow-release formulations of chlorpyrifos [*13–20*]. Other studies simulated pest control applications to rice fields [*21,22*]. All employed one to four applications at, or near, recommended rates, and biotic responses were observed for up to six months. Mortalities of fish, macroinvertebrates, or some zooplankton species were observed in most studies, algal responses were mixed, and molluscs and oligochaetes were unaffected. Direct comparison to laboratory studies is difficult because of the lack of precise data on toxicant concentrations in these field investigations, but the order of organism sensitivity is similar. Several reports are also available that describe responses of caged or free-living organisms following actual use applications of chlorpyrifos.

The single report of effects on organisms in natural streams resulted from two 15-min applications, one month apart, of 100 μg/L (unmeasured) to three Quebec rivers intended to evaluate blackfly control procedures [*23*]. Observations of responses, restricted to aquatic insects, indicated induction of a heavy drift of dead and dying nontarget insects as well as blackflies within 24 h after treatment. Post treatment benthic sampling showed that no species was completely eradicated.

Experimental Design, Materials and Methods

Facility

The present study was conducted at the Monticello Ecological Research Station, a field station of the U.S. EPA Environmental Research Laboratory at Duluth, MN. The station is located on 134 km^2 (13.4 hectares) of land leased from the Northern States Power Company and is adjacent to a nuclear electric generating plant and the Mississippi River at Monticello, MN. River water is continually pumped to a main laboratory building and then to eight outdoor experimental streams. The streams are each 520 m long with 1.4 km^2 (0.14 hectares) of water surface area and contain nine mud-bottomed pools alternating with eight gravel riffles. Pools are 30.5 m long, 3.6 m wide, and 76 to 86 cm deep, and riffles typically 30.5 m long, 2.4 m wide, and 10 to 20 cm deep. The facility is described further in reports of previous studies [*24–26*].

Three streams received a continuous, an intermittent, or no dosage (the control stream) of chlorpyrifos. River water flow into each stream was maintained at 200 gpm. Introduction of chlorpyrifos began on 19 May 1981 and terminated on 27 Aug. 1981, 100 days later. A 245 m reach near the upstream end of each stream, including four pools and four riffles, was used in the

biotic response part of the study (Fig. 1). The first pool in each stream (Section 2) was used to trap sediment in incoming river water by raising the water level with a wier at its downstream end. Three millimetre mesh stainless steel screens were positioned at the upper end of the first riffle and lower end of the second riffle to contain fathead minnows in stream Sections 3, 4, and 5. Bluegills were confined in Sections 7, 8, and 9 between 2.5-cm mesh galvanized iron screens 30 and 120 m below the fathead minnow reach. Polypropylene fabric shades supported by wooden A-frames straddled the first riffle, second pool, and second riffle of each stream. This reduced light intensity by 70 to 75% and limited macrophyte growth, thereby simplifying observation of organisms and sample collection. Chlorpyrifos concentrations for fate information were determined in samples taken along the entire length of each stream (16 sections).

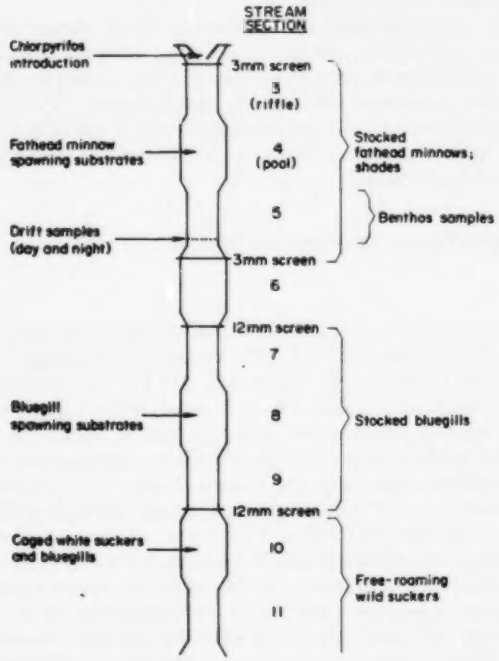

FIG. 1—*Diagram of the biological study area in each of the three experimental streams.*

Stream Dosing

Lorsban 4E®, an emulsifiable concentrate formulation of chlorpyrifos (0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate) produced by Dow Chemical Co., was used in the study. The container label specified active ingredients of 40.7% chlorpyrifos and 22.8% aromatic petroleum derivative solvent, and 36.5% inert ingredients. Stock solutions were obtained by mixing the emulsifiable concentrate with well water in 0.56-m^3 (150-gal) stainless steel tanks. The resulting slurry was continuously agitated vigorously with an electric mixer and pumped through polyvinyl chloride (PVC) tubing to the outflow from stream Section 2 (Fig. 1). Initially, stock solutions were prepared every two days for the continuous concentration stream, but it was discovered midway through the study that a stratification of the formulation was occurring in the stock tank, resulting in higher stream concentrations the first day than the second. Thereafter, new stock solutions were prepared every day. Stocks were pumped to the streams at rates of 30 to 180 mL/min depending on stock strength and desired stream concentrations. Stock solutions consisted of 2.2 to 23.4 mL of formulation mixed with 190 to 229 L of well water. Chlorpyrifos was pumped into the intermittently dosed streams for 24 h every 14 days, starting on the first study day. It was intended that the total amount of chlorpyrifos entering the water of each treated stream be equal, so a concentration approximately 14 times greater was pulsed into one stream for one day than was being continuously pumped into the other.

Initial toxicant concentrations used were based largely on the results of laboratory tests with *Crangonyx pseudogracilis*, the predominant amphipod in the streams. Twenty-four hour survival in toxicity tests was 45 and 50% at 3.5 and 2.8-μg chlorpyrifos/L so 3.1 μg/L was chosen as the pulse stream concentration and 0.22 μg/L the continuous concentration. These were raised two times during the study, the first time on 10 June (study Day 22) to compensate for an approximately 40% discrepancy in stream water concentration from that based on the calculated stock tank nominal. The second increase to nominal concentrations of 11.5-μg/L pulse and 1.01 continuous took place on 29 June (Day 41) because of a concern that the chlorpyrifos was not having a sufficiently severe impact to meet test objectives. The concentration in the continuous stock tank was reduced on 24 July (Day 66) to compensate for the increase in stream concentration resulting from a switch to daily stock preparation on 21 July.

Chlorpyrifos Analysis

Unless otherwise specified, chlorpyrifos concentrations are for unfiltered water samples taken several centimetres below the surface in the center of the various stream segments. Three samples were taken in one to three stream sections along the pulse stream immediately before each pulse (at noon) and

at hourly intervals for up to 6 h following each pulse. Further sampling in each of three stream sections occurred at 12, 24, 27, 36, 48, 51 h, and 4 and 7 days after each pulse for a total of 272 successful pulse stream analyses. A sample from the head, middle, and tail end of the continuous stream was taken nearly every test day for a total of 234 successful analyses. A total of 20 control stream samples were analyzed at regular intervals throughout the study. Each 225-mL water sample was stirred for 2 h with 25-mL petroleum ether in a 250-mL volumetric flask. One millilitre of extract was placed in a glass vial and quantitatively analyzed on a Hewlett Packard 5700 Series Gas Chromatograph (GC) with automatic sampler. The following GC conditions were employed: 6 ft, 2-mm column with 1.5% OV 17 + 1.95% QF-1 coating on 80/100 mesh Gas Chrom Q support; 200°C injection port, 190°C column, and 300°C detector temperatures; a mixture of 5% methane/95% argon carrier gas delivered at 23.3 mL/min; and a ^{63}Ni electron capture (EC) detector. The same technique was used for laboratory test water analyses except that hexane was the extractant.

Analytical quality assurance was accomplished by spiking a known concentration of chlorpyrifos in acetone into test water with every 12 samples to determine percent recovery; analysis of duplicate samples to determine technique variability; and cross-checks between the Duluth and Monticello laboratories to determine accuracy of measurements. A total of 65 spikes yielded an average recovery of 92% with a range of 53 to 118%, omitting the results from two brief periods when spiking solutions were faulty. Analysis of duplicates, within or between labs, indicated that measurement errors were approximately proportional to chlorpyrifos concentrations across the range measured (0.03 to 8.0 μg/L). The estimated standard deviation of the natural log of the measured concentration (approximately equal to the standard deviation of the percent error in the measurements) was 0.03 for chlorpyrifos extracted and then split between labs, 0.16 for samples split and then extracted, and 0.09 and 0.05 for duplicates analyzed within each lab. Duluth measurements were determined to be about 9% higher than corresponding values from Monticello. The mean and standard deviation of 52 spike recoveries run in conjunction with laboratory studies at Duluth were 102.35 and 16.2%. None of the chlorpyrifos exposure or fate concentrations reported below have been corrected for spike recoveries or interlaboratory differences.

The measured concentrations were used to fit continuous and pulsed concentration models to the data to describe the concentration of chlorpyrifos at all stream locations.[b] The average estimated in-stream concentrations (μg/L) for sections of the continuous concentration stream where biological effect measurements were made during five time periods corresponding to different dosages are shown in Table 1.

[b]Details of the stream concentration models can be obtained from the senior author upon request.

TABLE 1—*Average estimated continuous stream chlorpyrifos concentrations, μg/L.*

Time Period	Sampling Location (Stream Section)							
	3	4	5	6	7	8	9	10
5/19 to 6/9	0.14	0.14	0.13	0.13	0.13	0.12	0.12	0.12
6/10 to 6/28	0.15	0.15	0.14	0.14	0.13	0.13	0.13	0.12
6/29 to 7/20	0.33	0.32	0.31	0.30	0.29	0.28	0.27	0.26
7/21 to 7/23	0.83	0.80	0.78	0.75	0.73	0.70	0.69	0.66
7/24 to 8/27	0.47	0.46	0.44	0.43	0.42	0.41	0.39	0.38

For the pulse stream concentration model, three dosage periods were used to estimate the decrease in concentration going downstream. This descrease was found to be about 10% per section traveled, or at a somewhat faster rate than in the continuously dosed stream because of more rapid incorporation into sediments [*1*]. The model estimates of average in-stream concentrations, expressed in μg/L-day units under the time-concentration curves for each stream section are shown in Table 2.

These values integrate chlorpyrifos concentrations as the curves become wider (that is, take longer to pass a given point) and lower with distance traveled downstream. A poorly defined amount of the chlorpyrifos probably also desorbed from the sediments after the pulse peak had passed and therefore these are minimum concentrations which could even approach those occurring in Section 3. (If all of it desorbed, the difference in concentrations between sections would only be equal to the amount that degraded or evaporated.) Because of the difficulty in estimating the shape of the concentration versus time curve, the estimated values may have considerable error. Roughly, the estimates for Section 3 are best and unbiased, with a minimum relative error of 16% (95% confidence interval). All estimates probably have a relative error of less than 35%.

Figure 2 illustrates diagramatically the magnitude and duration of chlorpyrifos concentrations in the first riffle section (Number 3) of the pulse and continuously dosed streams. The total amounts of pesticide passing through the streams during the 100 study days can be calculated by converting all

TABLE 2—*Average estimated pulse stream chlorpyrifos concentrations, μg/L-day.*

Pulse	Date	Sampling Location (Stream Section)							
		3	4	5	6	7	8	9	10
1,2	5/9, 6/2	1.9	1.7	1.6	1.4	1.3	1.2	1.0	0.94
3	6/16	2.8	2.5	2.3	2.1	1.9	1.7	1.5	1.4
4–8	6/3, 7/14, 7/28, 8/11, 8/25	7.0	6.4	5.7	5.2	4.7	4.3	3.9	3.5

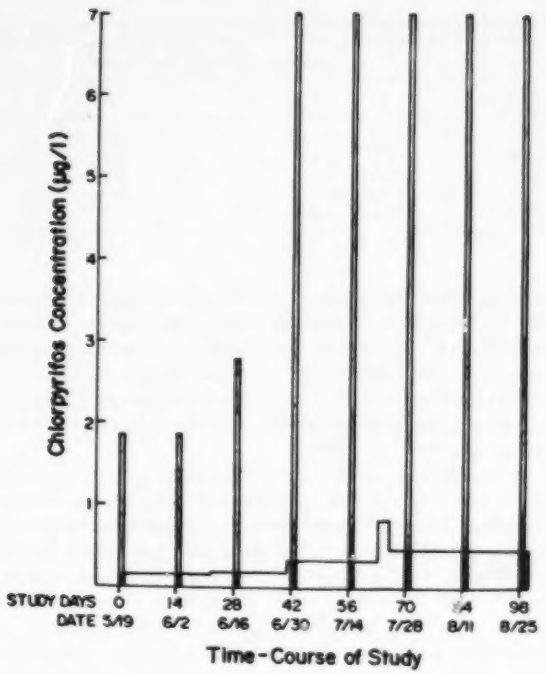

FIG. 2—*Model representation of chlorpyrifos concentrations in Section 3 of the pulse and continuous streams.*

values to µg/L-day units and summing them for each section. These are given in Table 3 for eight streams sections, along with the ratios of the totals in the two streams.

Here again, the ratios should be considered minimums and could approximate that for Section 3 in all downstream sections. These results indicate that the dosing scheme came reasonably close to meeting its intended objective of subjecting organisms in these two streams to equal amounts of chlorpyrifos.

The degradation of chlorpyrifos in both water and sediment was studied by Pritchard [*27*] under both formalin sterilized and biologically active conditions. The half-life of chlorpyrifos was determined to be 22 and 28 days, respectively, in water, and greater than 37 and 28 days in water and sediment. In addition, chlorpyrifos degradation was measured under stream conditions by taking a 4-L sample of water from the continuous concentration channel, setting it beside the stream under the polypropylene screen, and periodically

TABLE 3—*Total amounts of pesticide passing through streams for 100 study days calculated by converting all values to μg/L-day units and summing them for each section.*

Streams	Section							
	3	4	5	6	7	8	9	10
Continuous stream	31.6	30.7	29.8	28.9	28.0	27.1	26.4	25.6
Pulse stream	41.7	37.7	33.2	30.9	27.9	25.3	22.8	20.7
Ratio	1.32	1.23	1.11	1.06	1.00	0.93	0.86	0.81

analyzing samples for chlorpyrifos concentration. Under these conditions, the half-life of chlorpyrifos was about eleven days.

Two to four frozen fathead minnows or individual bluegills from a given day's sampling were prepared for tissue residue analysis by mixing the previously ground fish with anhydrous sodium sulfate in a blender. All fathead minnows and most bluegills had head and digestive tract removed before residue sample preparation leaving by weight 62 and 61%, respectively, of the intact fish. The blended sample was placed in a preweighed soxhlet thimble and extracted overnight with petroleum ether in a soxhlet apparatus. The extract was placed on a florisil column for lipid removal and was then eluted with 150 mL of 6% ethyl ether/94% petroleum ether. The mean percent lipid was 2.29% in 32 dissected fathead minnows and 0.48% in 12 dissected bluegills. The lipid concentration was about two times greater in 8 whole bluegills than in 12 dissected bluegills. The eluted tissue extract was concentrated by stream distillation before GC analysis. Duluth laboratory extractions used petroleum ether in an explosion-proof blender rather than with soxhlets. The same gas chromatograph column and conditions used for the water analyses were used for the fish tissue analyses except that the column temperature was programmed for 16 min at 190°C and 8 min at 240°C. Recoveries for 15 analyses of chlorpyrifos spiked into blended control fish samples had a mean of 71.6% and a standard deviation of 25.5%.

AChE Analysis

Fish brain acetylcholinesterase (AChE) activity was determined by the pH-stat method described by Jarvinen et al [*12*]. Most samples collected on a given date were pooled before analysis. Unfortunately, several samples intended for tissue residue and AChE analysis were destroyed by a freezer failure.

Water Quality

Several stream water quality characteristics were measured routinely throughout the study. Temperatures were automatically measured every 4 h

by thermocouples located at mid-depth in riffle Sections 3, 5, and 9 of each stream and at mid-depth and at the bottom of pool Sections 4 and 8 (Fig. 1). Weekly means were lowest (16.9°) in mid-May, peaked in early July at 25.4°, and decreased to 20.5° at the end of the study; weekly ranges averaged 4.1°. Weekly mean temperatures and ranges were usually within 0.2°C for mid-depth measurements in corresponding riffle and pool sections of the three streams. Weekly mean bottom temperatures were frequently 0.5 to 2.0°C lower than mid-depth measurements in the same pool. With five exceptions, weekly mid-depth means in Section 3 of all streams (45 measurements) were within 0.5°C of the weekly means in Section 9; only once was the difference greater than 1° (2.4°).

Hydrogen ion concentrations were also measured automatically with the probes located at mid-depth in Section 4 of each stream and in Section 8 of the continuous stream. Measurements of corresponding times were consistently within 0.2 pH units for the three streams. Weekly median minimums ranged from 7.7 to 8.4, and weekly median maximums ranged from 8.0 to 8.9. Weekly minimum values in Section 8 were 0.1 to 2.6 pH units lower and maximum values 0.1 to 2.2 pH units higher than minimum and maximum values in Section 4. The differences were largely attributable to plant metabolism.

Dissolved oxygen concentrations were measured over a substantial portion of the test period (6/5/81 through 8/12/81) as part of an investigation of possible effects of chlorpyrifos on production/respiration ratios [*28*]. Hourly measurements were obtained on 18 days by probes located in Sections 3 and 9 of each stream. Probes were calibrated daily using the Winkler titration method and a Yellow Springs Instrument Co. portable DO meter. Recorded DO values were corrected for calibration drift by assuming a linear change between daily checks. Water entering the streams usually contained DO concentrations above 75% of saturation, which became modified through metabolic activity with movement downstream. A summary of recorded DO values in mg/L is shown in Table 4.

Mean alkalinity, hardness, and conductivity obtained from three measurements during the study in each stream were 122 mg/L as calcium carbonate ($CaCO_3$), 140 mg/L as $CaCO_3$, and 276 μs, respectively. All were measured

TABLE 4—*Summary of recorded DO values, mg/L.*

	Control Stream		Pulse Stream		Continuous Stream	
Range and Values	Section 3	Section 9	Section 3	Section 9	Section 3	Section 9
Minimum daily range	1.02	4.38	1.42	5.45	1.37	4.46
Maximum daily range	3.72	7.87	3.44	8.77	3.02	9.38
Minimum value	4.20	2.53	4.20	2.52	5.02	2.33
Maximum value	8.60	11.56	8.86	12.11	8.27	11.71

according to techniques described in *Standard Methods* [28]. Variability between streams for a particular date and analysis was less than 10% of the mean; variability between dates in a specific stream was less than 20%.

Biology: Macroinvertebrates

The macroinvertebrate community was sampled for riffle benthos abundance and diversity, and organism drift to the downstream end of the second riffle (Fig. 1). Benthos were sampled in 14.3- by 12.8- by 15.0-cm deep stainless steel containers having 36-cm^2 screen-covered (0.6 mm mesh) openings on two opposing sides and a 0.02-m^2 open top. Three replicate samplers were set into each stream at 14-day intervals nine different times to provide a residence time of eight weeks before removal. The first set was removed five days before the first pulse and succeeding sets the day after termination of each pulse. Drift samples were collected by funneling stream water through nets with a mouth opening of 1350 cm^2 and a 90 cm long, 450-μm mesh Nitex bag. Nets were set for 1 h at 1300-h central daylight savings t[illegible] (the daytime sampling), 2200 h, and midnight (the nighttime sampl[illegible]gs). Daytime samples were collected in all streams for five consecutive day[illegible] only four days in three cases) that included two days before each pulse, [illegible] pulse day, and two days after each pulse, for a total of 37 samples per stream. Nighttime samples (both 2200 and 2400 h) were collected in all streams the day before, the day of, and the day after each pulse, plus, with one exception, a day midway between successive pulses for a total of 60 samples per stream.

Macroinvertebrates were washed from the benthic sampler gravel substrates and from drift nets with well water, collected on a 0.4-mm sieve, and preserved in 10% formalin containing 100-mg rose Bengal stain/L. Organisms were sorted and counted over a light box with the aid of a stereomicroscope. Subsampling procedures were used when more than 400 individuals of a given taxon were present in a sample. The more common organisms were identified to genus or species (leeches, isopods, amphipods, mayflies, *Cheumatopsyche* caddisflies, odonates, and snails) or family (springtails, caddisflies, Hemiptera, beetles, midges, and Ancylidae snails). Less common organisms were identified to order. Count data for various taxa were entered in a Lanier word processor and transferred via disk to the PDP 11/70 computer at Duluth for further processing. Data were analyzed on the basis of both number of organisms and number of taxa per sample in benthic containers ("cans") or drift. The early night (2200 h) drift samples were slightly larger but otherwise very similar to the late night (midnignt) samples both qualitatively and quantitatively, so the mean of these values is reported for a given date. Much of the extraneous variability in the data was removed by plotting the means of counts within eight different exposure periods, each period containing values for sampling dates closest to each of the eight pulse dates.

Biology: Fish

On 30 April 1981 600 juvenile fathead minnows were placed in the upper three study sections (riffle-pool-riffle) of each stream (Fig. 1). Stocking densities were based on the field observations by Smith et al [*29*]. The minnows were seined from a Hennepin County, MN lake by Ebner Bait Company, transferred to MERS holding tanks the next day, and stocked in the streams two days later. Sixty were randomly selected, anesthetized with MS-222, weighed, measured for standard length, and released into each stream.

About once a week at 1500 h, four Plexiglass® box traps (described by Zischke et al [*26*]) were placed in the minnow pool of each stream. They were removed at 0900 h the next morning and a maximum of 50 fish per stream were impartially selected for length measurement before returning them to the stream. Periodically, two to five minnows were measured, weighed, and immediately frozen for subsequent residue and brain AChE inhibition analysis.

During mid and late August the extent of deformed growth was quantified in fathead minnows from each stream. Trapped fish were observed in a shallow glass tray placed over a light box to estimate the percent deformed.

Spawning activity was determined every Monday, Wednesday, and Friday by randomly sampling 28 of 112 artificial spawning substrates (see Zischke et al [*26*]) placed in each minnow pool on 23 May (Fig. 1). Eight substrate columns were selected at random and embryos removed, counted by water volume displacement, and discarded. Subsamples were examined to estimate the number of dead embryos. Between 9 June and 17 July, estimates were also made of eyed embryos as a percentage of total embryos on substrates.

Adult bluegills from the National Fisheries Research Laboratory in La-Crosse, WI, were transferred to Monticello stream holding pens on 8 May 1981. On 12 May, 71 bluegills were stocked in a 90-m reach (Sections 7, 8, and 9), 30 m downstream from the fathead minnow section (Fig. 1). Stocking densities were based on the experimental ecosystem results of Wrenn et al [*30*]. All bluegills were anesthetized, measured, and randomly distributed between the three streams. Samples of fish for tissue residue, AChE inhibition, and ovary analysis were removed periodically by seining. The esophagus and stomach were removed and preserved in buffered formalin for food content determination.

Ten artificial spawning beds (gravel filled 33 cm long by 28 cm wide by 15 cm deep plastic dishpans) were placed in bluegill reach pools on 23 May (Fig. 1). Periodic observations were made throughout the spawning season to determine if these beds were being used and to mark and record the number of natural nests built. Between 29 June and 20 July all nests were sampled periodically with a metre long, 1.5-cm-diameter plastic tube. By sealing, releasing, and resealing the proximal end of the tube with the thumb, the distal end

of the tube placed on the nest surface collected material from about a 9-cm^2 nest area. This procedure was repeated five times on each nest or until sufficient numbers of embryos or larvae were collected to determine stage and condition. All embryos and larvae were placed in labeled containers, brought into the laboratory, and examined for viability.

Drifting fish larvae were collected in the same nets used to collect drifting invertebrates. Nets in all three streams were set for 1 h at 1300, 2200, and 2400 h (central daylight savings time) at the tail end of the lower riffle of the fathead minnow reach during the day of each pulse dose and 1, 7, and 13 days after each pulse dose. Similar nets were placed at the end of the lower riffle of the bluegill reach on the same days at 2300 and 2400 h. Net contents were rinsed into a pail, concentrated, and preserved in the same manner as macroinvertebrate samples.

On 3 and 4 Sept. the pools of stream Sections 2 through 8 were seined with a 3.18-mm (⅛-in.) mesh bag seine in an attempt to recover all fish. Consecutive seine hauls were made in each pool until the last haul contained less than 2% of the total recovered for that pool. All adult bluegills and random samples of the recovered fatheads were weighed, measured, and preserved in either formalin or ethanol. Nonstocked fish species caught in either the traps or seine hauls were counted, identified, measured, and discarded.

Laboratory and In-Situ Bioassays

In additon to the fathead minnow chronic toxicity test reported separately by Jarvinen et al [*12*], laboratory and field enclosure studies were conducted to compliment the 100-day stream study results. Amphipods collected for the laboratory tests were transferred to and maintained at the Duluth laboratory before use. They were cultured in tanks containing natural algal assemblages and fed well-soaked, autumn-dropped leaves and fish food. Flow-through tests were conducted in Lake Superior water in a cabinet containing a serial dilution apparatus [*31*], stock solution, and exposure chambers. The diluter was modified to deliver two concentrations that differed by a factor of ten. The flow rate was 900 mL/h to each 800-mL exposure chamber. All test solutions were prepared from the same chlorpyrifos formulation (Lorsban 4E®) used in the streams. Ten amphipods were placed in each of the duplicate treatment chambers (including two controls) and exposed either continuously for 8 days, or for 24 h before being removed to chlorpyrifos-free water for 7 days. Animals in each container were checked daily for survival and other effects. Water temperature during the tests was 21.8 ± 0.5°C.

In the field enclosure tests at Monticello, amphipods were collected from the control stream and exposed in the chronic and pulse streams in May and June. Exposure chambers were stainless steel baskets fitted with a Styro-

foam® collar. The collar-fitted baskets were attached to a bridge in Section 3 by a cord and floated in mid-stream. Each stream contained duplicate baskets with each basket containing ten randomly assigned amphipods. Total exposure or observation time or both did not exceed 48 h.

Fifteen adult bluegills from Pleasant Lake, Wright County, were placed in wood framed polypropylene mesh cages (0.9 by 1.4 by 0.9 m) in the upstream end of the pool immediately downstream from the bluegill section in each stream (Fig. 1) from 21 May to 27 Aug. These provided an easily observed group in which mortality, especially, could be documented if it occurred. All caged fish obtained their own food. Because stressed and dying white suckers were observed after Pulse 5 on 14 July in the pulse stream immediately below the lower bluegill barrier, 15 white suckers, obtained from a local bait dealer, were placed in cages in Section 10 of each stream on 21 July. Two fish were removed from each cage for AChE analysis before and after Pulse 6 on 28 July and from the cages in the control and continuous streams on 28 Aug. All caged suckers in the pulse stream died before 28 Aug.

Results

Macroinvertebrates

The relative abundance of dominant organisms and total numbers of organisms in the riffles of the three streams were considerably different at the start of the study (Table 5). The differences between streams were larger than can be explained by the sampling variability and any associated measurement errors. This lack of close agreement persisted throughout the study with the total numbers and trends in numbers being closest at the end of the test. Riffles in the two streams receiving chlorpyrifos rapidly became dominated by isopods while the control stream, less rapidly, evolved from an isopod-dominated to an amphipod-dominated riffle benthos.

Day drift of invertebrates in the first half of the study was about half the magnitude of nighttime drift (Tables 6 and 7). Amphipod drift was relatively high during the day in the continuously treated stream but occurred mostly at night in the control stream. The peak amphipod drift in the continuous stream appears to correspond to a drastic reduction in benthic amphipods during the fourth exposure period when chlorpyrifos concentrations were approximately doubled from their previous levels (from 0.15 to 0.33 μg/L in stream Section 3). In the second half of the study, day and night drift were both low in the chlorpyrifos treated streams but remained high at night in the control streams.

The mean number of invertebrates per sample in the nighttime drift in the pulse stream, which contained very few amphipods, decreased significantly ($P = 0.05$) from the day before or the day of pulses to the day after pulses.

TABLE 5—*Relative abundance (as percentages) of major taxa, and total numbers of organisms, per riffle benthos sample. Table values represent the mean of all samples collected nearest to each pulse date; blanks indicate total absence of that taxon.*

Channel and Taxon	Exposure Period, Percentage of Total Macroinvertebrates							
	1	2	3	4	5	6	7	8
CONTROL STREAM								
Amphipods	6.0	36.9	41.6	44.9	59.7	52.3	74.4	81.8
Snails	1.9	11.3	3.3	4.9	4.6	13.9	7.3	1.3
Chironomidae	3.9	6.2	10.5	3.9	2.3	2.4	0.5	1.0
Asellus isopods	85.7	42.6	37.8	37.9	28.1	13.3	7.4	4.8
Turbellaria	1.4	2.0	5.9	6.5	4.9	15.6	9.9	9.5
Total numbers of all macroinvertebrates	5705	5986	4344	4103	3812	1677	2661	3344
PULSE DOSE STREAM								
Amphipods	0.1	1.0	0.0	0.0	0.0	...	...	...
Snails	0.3	16.6	6.0	3.7	5.8	4.5	5.1	5.4
Chironomidae	66.9	0.2	0.1	0.0	0.0	...	...	...
Asellus isopods	13.6	72.8	86.5	88.8	89.2	87.7	82.6	84.7
Turbellaria	14.1	7.2	7.0	7.3	4.9	6.6	11.2	9.8
Total numbers of all macroinvertebrates	1037	1205	3776	6863	7020	5112	3442	3000
CONTINUOUS DOSE STREAM								
Amphipods	69.1	64.0	52.9	8.3	4.6	...	...	0.0
Snails	0.3	0.9	0.3	2.4	0.9	1.5	0.8	0.6
Chironomidae	12.9	0.1	0.1	0.0	0.0	...	...	...
Asellus isopods	14.0	31.5	43.8	76.1	79.0	88.5	88.9	90.3
Turbellaria	0.6	3.1	2.7	9.7	15.0	9.6	10.1	9.0
Total numbers of all macroinvertebrates	2720	4963	4872	2877	4492	3611	3196	4991

Daytime mean drift following pulses was also lower but not significantly so. No such changes occurred on these dates in the control or continuously dosed streams.

Macroinvertebrate data were also analyzed for the number of different taxa identified per benthos or drift sample. In general, the shapes of the trend curves are similar for benthos, daytime drift, and nighttime drift (Fig. 3). The mean number of taxa per sample was highest for all streams in the nighttime drift. The overall mean number of taxa was significantly ($P = 0.05$) lower in the chlorpyrifos-dosed streams than in the control streams and declined fastest in the second half of the study in the nighttime drift.

A significant ($P = 0.05$) drop in the mean number of taxa occurred in the pulse stream nighttime drift in the 24 h after pulses as compared to the 24 h before pulses. This change was not observed in the other two streams on these dates and corresponds to the drop in mean numbers of organisms of all taxa 24 h after pulses.

Two exposures of amphipods in floating baskets took place in the pulse

TABLE 6—*Relative abundance (as percentage) of major taxa, and total numbers of organisms, per daytime (1300 h) drift sample. Table values represent the mean of all samples collected nearest to each pulse date.*

Channel and Taxon	Exposure Period. Percentage of Total Macroinvertebrates							
	1	2	3	4	5	6	7	8
CONTROL STREAM								
Amphipods	25.9	5.8	60.3	51.1	27.5	20.4	27.5	18.4
Snails	16.7	39.2	8.2	18.1	48.1	44.8	17.8	7.7
Chironomidae	25.5	2.1	1.3	2.8	4.2	1.2	23.2	50.0
Asellus isopods	4.1	3.1	4.1	5.5	4.8	2.1	8.7	2.1
Turbellaria	1.4	0.3	1.0	8.8	8.4	3.0	4.8	4.7
Total numbers of all macroinvertebrates	145.2	793.4	171.6	115.8	47.3	82.0	78.6	58.5
PULSE DOSE STREAM								
Amphipods	2.4	0.9	4.5	1.7	2.6	0.5	0.7	0.3
Snails	17.7	94.2	81.0	90.1	79.7	81.7	66.7	33.8
Chironomidae	25.3	0.3	0.3	0.2	0.7	1.0	1.8	3.1
Asellus isopods	0.6	0.7	0.6	2.4	4.1	1.4	5.9	5.2
Turbellaria	0.3	0.2	1.3	0.4	8.3	13.7	23.6	5.7
Total numbers of all macroinvertebrates	287.4	2938.6	1218.6	597.0	135.8	303.7	276.6	327.0
CONTINUOUS DOSE STREAM								
Amphipods	87.1	42.9	73.3	96.0	82.5	62.0	7.9	5.1
Snails	0.5	35.0	3.3	1.3	4.8	16.6	27.6	4.6
Chironomidae	5.9	0.7	0.7	0.0	0.4	1.7	3.3	6.3
Asellus isopods	0.3	3.1	1.9	0.3	1.9	9.2	23.7	6.3
Turbellaria	0.1	1.8	0.8	0.1	1.1	11.0	36.2	21.1
Total numbers of all macroinvertebrates	1003.6	4	491.4	2669.6	270.3	57.3	30.4	43.8

dose stream. Mean measured chlorpyrifos concentrations during the pulses were 1.7 μg/L (on 19 and 20 May) and 2.5 μg/L (on 16 and 17 June). At 22 and 26 h after the start of the first pulse, 50 and 55% of the amphipods were immobilized or killed. During the June pulse, 40 and 65% were immobilized after 24 and 48 h of exposure. A single two-day exposure in the continuously dosed stream on 16 to 18 June to a calculated concentration of 0.15 μg/L produced no amphipod mortalities.

Amphipod laboratory toxicity test results were similar to in-stream results (Table 8). Four animals (20%) exposed continuously to 0.55 μg/L were dead after 24 h, and 8 of the remaining 16 animals could not swim away from a gentle prodding. Those that survived beyond four days all became immobilized. Results of the pulse exposure indicate that the mortality rate is about the same as in the lower concentration continuous exposure, and that death continues to occur for several days beyond the exposure period. In the pulse exposure also, those animals surviving were immobilized by the end of the test.

TABLE 7— *Relative abundance (as percentages) of major taxa, and total numbers of organisms, for 2200 h and midnight drift samples combined. Table values represent the mean of all samples collected nearest to each pulse date; blanks indicate total absence of that taxon.*

Channel and Taxon	Exposure Period, Percentage of Total Macroinvertebrates							
	1	2	3	4	5	6	7	8
	CONTROL STREAM							
Amphipods	25.9	73.3	90.5	95.4	78.0	72.6	85.3	78.9
Snails	3.8	8.8	1.6	1.8	6.2	18.9	7.2	2.5
Chironomidae	20.5	0.7	0.2	0.1	0.3	0.8	2.5	4.2
Asellus isopods	3.0	0.4	0.4	0.2	0.2	0.1	0.0	0.1
Turbellaria	0.3	0.2	0.4	0.6	0.7	0.5	1.0	0.6
Total numbers of all macroinvertebrates	270.2	2306.0	1542.1	1379.8	714.1	2169.3	847.2	822.2
	PULSE DOSE STREAM							
Amphipods	1.5	2.4	3.8	3.2	4.0	3.2	0.3	0.8
Snails	3.4	73.0	64.2	79.8	54.6	61.2	61.8	52.4
Chironomidae	19.3	0.4	2.2	0.0	0.6	1.0	1.0	4.7
Asellus isopods	0.1	0.4	0.8	2.3	6.8	4.4	3.2	16.7
Turbellaria	0.1	0.2	1.8	2.6	7.5	18.0	29.4	16.6
Total numbers of all macroinvertebrates	407.5	1581.3	1742.9	734.5	174.9	151.8	186.3	148.5
	CONTINUOUS DOSE STREAM							
Amphipods	76.6	93.5	86.9	96.1	78.6	71.0	33.1	35.0
Snails	0.2	1.5	0.4	0.5	3.2	9.6	11.5	4.7
Chironomidae	6.0	0.1	0.2	0.0	0.1	0.1	0.7	...
Asellus isopods	0.1	0.0	0.3	0.2	1.4	6.9	9.3	14.0
Turbellaria	0.0	0.0	0.4	0.2	1.5	3.3	33.4	15.2
Total numbers of all macroinvertebrates	1662.7	4292.0	2553.9	4236.7	415.5	183.0	59.2	42.8

Fathead Minnows

A 23.6 and 19.4% incidence of deformed fathead minnows (usually a slight or moderate bending of the caudal region [scoliosis] or a shortening of the caudal region) in the pulse stream was detected in the 18 and 26 Aug. samples, respectively (Table 9). Minnows from the control and continuously dosed streams had 2.0% or less deformities. A higher percent of deformities occurred in the second generation than in the first in the pulse stream.

No other effects of chlorpyrifos on survival, growth, or reproduction of fathead minnows or bluegills were observed during the study. First generation fathead minnows weighed an average of 2.9, 2.7, and 3.2 g at termination in the control, pulse, and continuous streams, an overall mean weight gain of 2.0 g each. Condition factors $W(g) \times 10^5/L^3$ (mm) [*32*] were similar in all three streams and followed a general trend: low in early spring, high at spawning onset and early spawning, variable during midspawning, and low during late spawning. Natural mortality during the study (not counting those

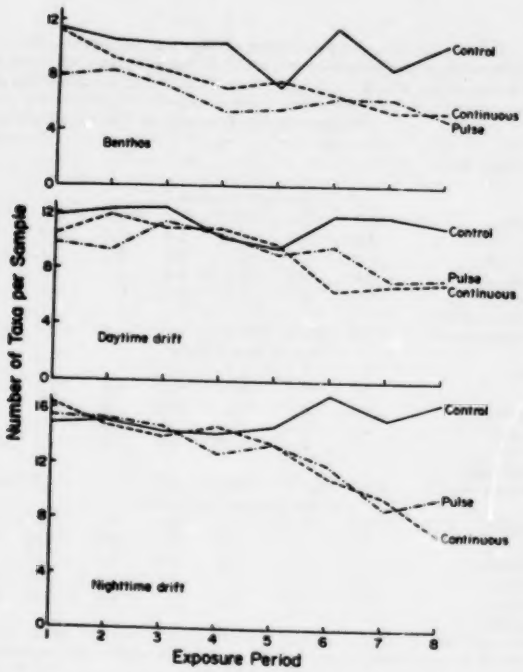

FIG. 3—*Mean number of taxa per riffle benthos, daytime drift, and nighttime drift sample during each of eight exposure periods.*

TABLE 8—*Mortality of amphipods in laboratory flow-through exposures to chlorpyrifos.*

A. Continuous Exposure to 0.55-μg Chlorpyrifos/L			B. Pulsed Exposure for 24 h to 2.8 μg/L (Test 1) and 3.7 μg/L (Test 2)[a]		
Exposure Duration, h	Exposure Mortality, %	Control Mortality, %	Observation Period, h	Exposure Mortality, %	
				Test 1	Test 2
24	20	0	24 (exposure)	20	0
48	40	0	48	50	55
72	-65	10	72	55	70
96	80	10	96	60	70
192	95	20	120	60	80
			144	60	85
			168	...	85

[a]Control mortality was 5% in each test.

TABLE 9—*Deformed growth of fathead minnows sampled after the 7th and 8th chlorpyrifos pulse (13th and 14th week of the experiment).*

Stream	1st Generation			2nd Generation[a]			Both Generations		
	Sample Size	Number Deformed	Deformed, %	Sample Size	Number Deformed	Deformed, %	Sample Size	Number Deformed	Deformed, %
					Aug. 18				
Control	49	1	2.0	0	...	...	49	1	2.0
Pulse	60	10	16.7	12	7	58.3	72	17	23.6
Continuous	63	0	0	7	0	0	70	0	0
					Aug. 26				
Control	149	1	0.7	1	0	0	150	1	0.7
Pulse	191	26	13.6	108	32	29.7	299	58	19.4
Continuous	6	0	0	1	0	0	7	0	0

[a]Fish ≤ 50 mm were considered to be 2nd generation.

removed for tissue and enzyme analysis) was 47, 39, and 45% for the control, pulse and continuous streams, respectively. Instantaneous growth rates G mean standing stock $\bar{B}$, and production ($P = G\bar{B}$) were calculated according to Ricker [53]. Production (total weight) values of 909.4, 920.4, and 1049.9 g were obtained for the control, pulsed, and continuous stream, respectively, or 2% higher in the pulse and 19% higher in the continuous chlorpyrifos streams than in the control stream.

Spawning began in the pulse and continuous streams during the third week of May and about a week later in the control stream. Embryo deposition on artificial substrates in the continuous stream was 48% higher than the control, and in the pulse stream was 13% less than the control. Mean number of embryos per utilized substrate in the continuous stream was 6% higher than the control, and in the pulse stream was 9% lower than the control. Estimated dead as a percent of total production were 0.11, 0.14, and 0.21% in the control, pulse and continuous streams, respectively. Most of the dead embryos in the pulse and continuous streams were found during the first week of spawning. Estimated "eyed embryos" as a percentage of embryo production from 9 June through 17 July were 42, 34, and 44% in the control, pulsed, and continuous streams, respectively. Daily percentages ranged from 0 to 100.

Drifting fathead minnow larvae (nearly all were 4 to 6 mm long) in the continuous and pulse streams exceeded that for the control by 1.97 and 3.17 times, respectively. As an index of reproductive success, these numbers do not correlate with the embryo production numbers. Late July and August drifts, after cessation of embryo production on the artificial substrates, indicate that the males shifted their spawning to natural substrates. Embryos were observed in mid-July on partially submerged broadleaf plants such as *Polygonum* and *Sagittaria*, which were immature and unavailable as substrates in May through about mid June.

More than 80% of the drift in the fathead minnow section of all three streams occurred during the 2200-h sampling period, whereas the drift in the bluegill section was more equally distributed between the 2200 and 2400 h collections. No change in drift densities could be detected in the pulse stream during and after the pulse dose when compared to drift densities in the control and continuously dosed streams.

The number and length frequencies of the fathead minnows recovered from the three streams showed that 60 mm is a good estimate for demarcation between the two generations, that the abundance and size of first generation fish were similar in all three streams, and that the abundances of second generation fish were strikingly different (Fig. 4). The total number of second generation minnows captured in the continuous stream was 69 times greater than from the control stream; in the pulse stream 13 times more fish were removed than from the control. The trend in abundance of second generation fish from succeeding downstream pools was similar in the continuous and pulse streams.

Bluegills

Growth of bluegills was similar in all three channels for the 93 days between stocking on 12 May and recovery on 13 Aug. Control bluegills averaged 116 g,

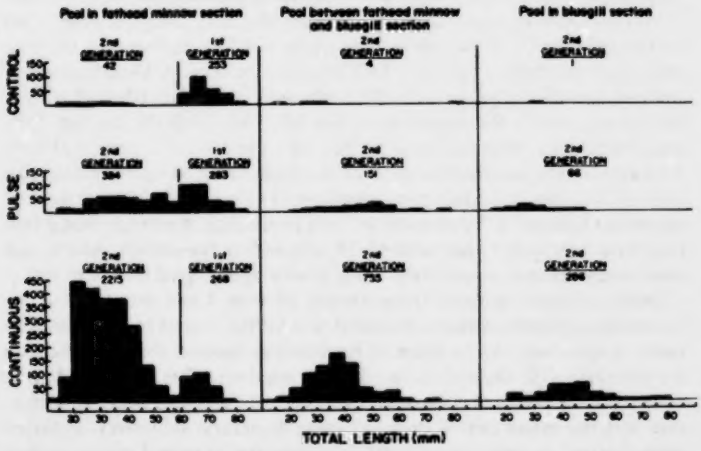

FIG. 4—*Number and size distribution of fathead minnows recovered from stream pools on 3 Sept. 1981.*

and those from the pulse and continuous streams averaged 120 and 115 g, respectively. Weight gains ranged from 30.0 to 31.6 g and were not different between streams. Of the total of 71 bluegills stocked in each stream, about 40% were removed for tissue residue and enzyme analysis, 30% were recovered dead, 25% were not accounted for, and 5% were captured alive at termination.

Lack of a chlorpyrifos effect on survival is supported by a low mortality among caged bluegills (two fish died in the control stream and one fish in each of the chlorpyrifos-dosed streams). However, the caged bluegills in the pulse stream were lethargic, and some had tetanic spasms immediately following the 24-h doses. Neither symptom was noted in caged fish in the control or continuously dosed stream at any time during the exposure, and the symptoms disappeared two to four days after each pulse. On 13 Aug., two days after the second to last pulse, samples of free roaming bluegills collected in the control and continuously dosed streams were active and appeared to be in good condition; those collected in the pulse stream had the same healthy color and plumpness, but they were lethargic and some had tetanic spasms (similar to the pulse stream caged bluegills) when handled.

Spawning occurred in all three streams. The first nest was observed on 4 June in the pulse stream, and by 12 June five to ten clustered nests were found in each stream. The total number of active nests through 20 July was six (four natural, two artificial) in the control, four (three natural, one artificial) in the pulse stream and four (two artificial, two natural) in the continuous stream. All embryos and larvae sampled from the nests in all three streams appeared to be in good condition. The pools in each of the three streams became so full of filamentous algae after 17 July that further observation of nests was not possible. This may have been near the end of the spawning season as larval drift diminished after mid-July. Total number of drifting larvae was greatest in the control stream and least in the continuously dosed stream, but because of the large variation (for example, 50 to 60% of the season total in all three streams were caught in one sample) little or no significance could be attributed to differences between streams. Likewise because of the high variation, no conclusions were possible regarding differences between streams in the drift frequencies according to the hours of day or the days during and following the pulse dose.

As shown in Table 10, adult bluegills had diverse feeding habits. Nearly all macroinvertebrates and plant types found in the streams were represented in the stomach contents. Large numbers of chironomid larvae consumed on 20 and 21 May in all three streams correspond to the peak numbers found in the streams just before those dates. During late July and August the dominant food items were isopods and plants in the control and pluse streams and a greater diversity of foods (algae, cladocerans, odonates, Lemna, isopods, and so forth) in the continuous stream.

TABLE 10—*Stomach analysis of adult bluegills in the control, pulse, and continuously dosed streams.*

Date	Channel	Number of Fish	With Stomach Contents	Estimated Wet Volume in Stomach, %			Mean Number of Identified Organisms per Fish Stomach[a]									
				Fil. Algae	Plant Fragments	Unidentified Fragments	Physid Snail	Cladoceran	Isopod	Amphipod	Odonata Larva	Coleoptera Larva	Chironomid Larva	Chironomid Adult	*Lemna minor*	Other[b]
May 20 and 21	control	4	3	5.0	0	58.3	0	0	0	0	0	0	30.7	0.3	0	0.7
	pulse	4	3	0	0	25.0	0.7	0	1.7	0	0	7.7	115.0	4.7	0	1.4
	continuous	2	2	0	0	0	0	63.0	0	0.5	0	1.5	537.0	1.5	0	0.5
June 27 to Aug. 4	control	10	10	22.0	0	31.5	0.1	0.1	0.4	0.2	1.8	0.2	0.2	0	11.3	0.7
	pulse	7	4	2.5	0	50.0	0.5	0	4.5	0	0	0	0	0	0	0.8
	continuous	10	8	0.6	0	54.4	0.1	0	6.9	1.9	0	0	0.1	0	0.6	1.2
Aug. 13 and 26	control	8	7	15.0	6.4	27.7	1.7	88.6	5.7	1.1	0.3	0	0.1	0.1	0	2.7
	pulse	8	4	22.5	5.0	57.5	0	0	1.0	0	0	0	0.3	0	0	0
	continuous	6	6	0	6.6	19.2	0.2	0	11.7	0.3	0	0	0	0	0	0.4

[a]Mean includes only fish with stomach contents.

[b]Other includes: May 20 and 21 control-lepidoptera larve; pulse-sphearid clam and corixid adult; continuous-annelid. July 27 to Aug. 4 control-lepidoptera and tricoptera larva, coleoptera and hemiptera (corixid) adult; pulse-sphearid clam and corixid adult; continuous-tricoptera larva, coleoptera and hemiptera (belostomid) adult, leech. Aug. 13 control-lepidoptera, tricoptera and ephemeroptera larva, hymenoptera adult, leech, copepod; continuous-coleoptera adult, corixid adult.

Other Fishes

Fourteen other fish species entered the streams from either the intake or discharge. Most were captured either above the upstream fathead barrier or below the bluegill downstream barrier during the final recovery on 3 Sept. None were captured in the bluegill sections. A smallmouth bass and two burbot were captured in traps in the fathead section of the control stream in early and mid-June.

Of the nonstocked species only white suckers were found dead or dying in any of the streams. During the fifth pulse in mid-July, several white suckers immediately downstream from the lower bluegill barrier were found dead or exhibited symptoms similar to those of caged bluegills—stiff quivering body with pectoral fins in a rigid outward position. The eleven caged white suckers placed in the streams on 21 July were also affected by the next pulse on 28 July. Nine fish in the pulse stream were immobilized and severely distressed on 29 July, three died by 30 July, and the rest were very lethargic. One of eleven fish also died in the continuous stream by 30 July, and this group appeared generally "less active" than the controls. By 31 July, the remaining pulse stream fish appeared largely recovered.

Tissue Residue and AChE Analyses

Bioconcentration factors were similar at different exposure concentrations as indicated by the fathead minnow continuous stream bioconcentration factors (BCF) results (Table 11), so pulse stream results were combined for the same time intervals after the start of each pulse. Tissue concentrations in the pulse stream are therefore expressed as a percentage of that found on Day 1 (fish were collected just before termination of a given 24-h pulse). Accumulation was rapid, BCF's after about 20-h pulse exposure approaching those observed in the continuously dosed stream. Tissue concentrations dropped about 50% per day after pulse termination and reached a low plateau or slowly diminishing level by six days after. Expressing the results as lipid BCF reduces concentration and species differences and indicates an affinity of chlorpyrifos for lipids.

The nature of the AChE pulse stream data indicated that combining data for 21 and 45 h after initiation of a pulse and 7 to 13 days after was appropriate (Table 12). The percent inhibition of brain AChE activity for both fathead minnows and bluegills was greater following pulses than after exposure to corresponding (14 times less) continuous concentrations. Fatheads and bluegills appeared to be about equally sensitive to chlorpyrifos inhibition. The extent of recovery between pulses tended to be inversely related to the maximum degree of inhibition. White suckers appeared to be more vulnerable to AChE inhibition than the other two species analyzed.

TABLE 11—*Chlorpyrifos tissue residues and bioconcentration factors (BCF) for fathead minnows and bluegills in the pulse and continuously dosed streams.*

Day Since Start of Pulse[a]	$\bar{X}$ Water Concentration, µg/L	% of Day 1 Tissue Concentration	Number of Fish	Tissue BCF	% of Day 1 Lipid Concentration	Number of Fish	Lipid BCF	Exposure Period	$\bar{X}$ Water Concentration, µg/L	$\bar{X}$ Tissue Concentration, µg/kg	Number of Fish	Tissue BCF	$\bar{X}$ Lipid Concentration, µg/kg	Number of Fish	Lipid BCF
Fathead Minnows, Pulse Stream								**Fathead Minnows, Continuous Stream**							
1	*b*	100	21	400	100	2	8700	5/19-6/9	0.14	80	20	570	2800	4	20000
2	*b*	55	9					6/10-6/28	0.15	39	8	260	2900	4	19300
4	*b*	16	9					6/29-7/20	0.32	250	6	780			
7 and 8	*b*	1.3	9		2.6	2		7/24-8/27	0.46	350	14	760	10700	12	23000
14	*b*	0.8	23		0.3	4		X, all periods				590			21400
Bluegills, Pulse Stream								**Bluegills, Continuous Stream[d]**							
1	*c*	100	2	110				7/24-8/27	0.41	42	13	100	5000	13	12000
2 and 3	*c*	37	16												
7	*c*	2.6	2												
13 and 14	*c*	2.0	4												

[a]Data for a given day after pulse combined for all pulse dates and $\bar{X}$ water concentrations; fatheads actually collected 3 h earlier than the time (in day) indicated.
[b]Data combined for three 24-h pulses at 1.7, 2.5, and 6.4 µg/L.
[c]Data combined for two 24-h pulses at 1.2 and 4.3 µg/L.
[d]Results combined for whole and for headed and gutted bluegills.

TABLE 12—*Acetylcholinesterase enzyme inhibition analyses.*

Stream	Sample Collection	Number of Fish	$\bar{X}$ Chlorpyrifos Concentration	$\bar{X}$ Percent of Control Stream Activity
		FATHEAD MINNOWS		
Pulse	1 to 2 days after pulse[a]	4	1.7	55
Pulse	7 to 13 days after pulse	14	1.7	88
Pulse	1 to 2 days after pulse[a]	4	2.5	56
Pulse	7 to 13 days after pulse	4	2.5	93
Pulse	1 to 2 days after pulse[a]	20	6.4	31
Pulse	7 to 13 days after pulse	20	6.4	73
Continuous	5/20 to 6/17	27	0.14[b]	76
Continuous	6/30 to 8/26	28	0.42[b]	59
Control	5/20 to 8/26	49	0	...
		BLUEGILLS		
Pulse	1 to 2 days after pulse[a]	2	1.2	84
Pulse	1 to 2 days after pulse[a]	13	4.3	40
Pulse	7 to 13 days after pulse	6	4.3	84
Continuous	5/20	2	0.12	72
Continuous	7/27 to 8/13	8	0.41	60
Control	5/20 to 8/26	17	0	...
		WHITE SUCKERS		
Continuous	7/27 to 8/25	7	0.38	41
Control	7/27 to 8/25	4	0	...

[a]Fish actually removed from pulse stream 21 and 45 h after initiation of pulse.
[b]Time-weighted average concentration during sample collection period indicated.

Conclusions

Little if any impact of chlorpyrifos on organism total abundance was indicated by the benthic sampling results. The control stream contained more organisms at the start and was intermediate at the end. There were, however, major changes in the numbers of dominant species.

Several types of evidence indicate that chlorpyrifos was responsible for the shift from amphipods to isopods as the dominant organism in the continuous stream. Amphipods maintained a strong population throughout the study in the control stream, being the most numerous organism for most of that time while they disappeared almost totally in the dosed streams. The laboratory data indicated that all amphipods were killed or immobilized in eight days at a chlorpyrifos concentration (0.55 μg/L) very close to the mean concentration (0.50 μg/L) for Section 3 of the continuous stream for the final 37 test days. The stream concentration was 0.33 μg/L in Section 3 during the preceding 22 days. The much higher levels of drift by amphipods in the continuous as compared to the control stream in the first half of the experiment might indicate an avoidance or escape reaction. The very low nighttime drift thereafter undoubtedly reflects a very low population density. Amphipod drift in the continuously dosed stream was particularly high during exposure Period 4 when

the exposure concentration was approximately doubled. Arthur et al [*25*] also observed elevated daytime drifts of amphipods following two- to threefold increases in continuous concentrations of the organophosphorous pesticide diazinon. Daytime drift in the control stream of the present study was consistently low, which is in agreement with previous observations of normal diel drift patterns.

Chironomids were abundant in the benthos of all three streams at the beginning of the study but became greatly reduced in both chlorpyrifos-dosed streams by the second exposure period. The number in the control stream remained relatively stable through the third exposure period and then gradually declined. An elevated nighttime drift during the third exposure period in the pulse stream, which appears high in relation to benthic abundance, might indicate a toxicant induced emigration. These possible indications of effect are questionable, however, because the density of chironomids in the control streams of previous studies [*24–26*] also dropped rapidly in early to mid June as a result of emergence. A slight recovery in numbers was observed in the control stream in this and previous studies in late August but did not occur in either of the chlorpyrifos-dosed streams further suggesting a chlorpyrifos effect. Laboratory exposures have produced EC_{50} values down to 0.5 μg/L for larval chironomid life stages [*34*].

No adverse effect was observed on the other three dominant taxa-isopods, turbellarians, or snails. The relative sensitivity of these organisms, and also amphipods, to chlorpyrifos is similar to their sensitivity to diazinon [*25*]. The daytime drift of snails observed in the pulse and control streams is not unusual (J. A. Zischke, St. Olaf College, Northfield, MN, personal communication) nor is the timing of peak abundance observed in the pulse stream in May and June [*24–26*]. The present study also concurs with the others in regard to the predominance of amphipods over isopods during most of the summer in the control streams. The consistent success of isopods in the near absence of amphipods might indicate that the two taxa compete with one another for resources.

The significant decrease in pulse stream nighttime drift the day after pulses contrasts interestingly with the observations by Wallace et al [*23*] of very large increases in drift during the 24 h following a 15-min dose period at 100-μg chlorpyrifos/L. Muirhead-Thomson [*35*] also found *Gammarus* amphipods and *Baetis* mayflies to be "activated" to drift downstream after exposure to 100-μg chlorpyrifos/L for 30 min, a concentration resulting in 100% mortality in 24 h. One explanation for the decreased drift in the present study is that the organisms initially became less active in response to the much lower concentrations. Another explanation is that snails, the most numerous invertebrates in the pulse stream, react differently to chlorpyrifos than other invertebrates. As mentioned previously, the increased day and night drift after test Day 40 (Period 4) in the continuous stream could very well have been stimulated by the doubling of the chlorpyrifos concentration.

The diversity of the macroinvertebrate community, as measured by the number of taxa in benthic and drift samples, declined about equally in both chlorpyrifos-dosed streams as compared to the control stream. The decline appeared from benthic analysis to begin near the start of the study, but was not apparent in drift samples until the last three exposure periods. The most significant changes were observed in night drift samples where the greatest number of taxa was recorded. The downward trend in macroinvertebrate diversity in the dosed streams would probably have continued if the study duration had been longer. No individual taxa disappeared completely but instead were observed less frequently. The lack of total disappearance might be due to incursion of sensitive taxa along with the water from the Mississippi River, or perhaps because of remnants of populations existing in microhabitats with lower chlorpyrifos concentrations. A third possibility for some taxa is that surviving individuals were more tolerant of chlorpyrifos than the others. While diversity appears to be a more valuable indicator of effect than organism density in this study, Arthur et al [*25*] found the opposite to be true with the pesticide diazinon.

In general, the effects on the macroinvertebrate communities in the pulse and continuously dosed streams appear similar and, therefore, indicate a comparable sensitivity to the average chlorpyrifos concentration, whether it is continuous or intermittent in nature. Differences in the abundance of dominant species at the start of the study do not preclude such a conclusion. Still, it is possible that the presence or prevalence of a single taxon, perhaps one considered "important," might be dependent on the nature of various exposures (total amounts of toxicant being equal) even if community level responses (for example, species diversity) to the agent were the same. Therefore, a conclusion about whether effects of different exposures were comparable might depend upon which results were cited or which parameters were studied.

The only definite chlorpyrifos effect on fathead minnows was an increased incidence of spinal deformity first observed after the seventh pulse in the pulse stream. This effect has occurred frequently in various fish species exposed both acutely [*36*] and chronically [*12,37–39*] to organophosphate pesticides. No adverse effects were seen among continuously dosed stream fathead minnows indicating that the impact of chlorpyrifos in the stream was probably no greater than on fathead minnows in the laboratory life-cycle chronic test conducted by Jarvinen et al [*12*]. The two data sets are not easily compared because the laboratory study lasted longer (200 days total), started with newly hatched fish, and allowed more precise quantitation of effects on reproduction. Reproduction comparisons are especially tenuous because of the short exposure period before spawning in the streams. Jarvinen et al [*12*] saw first generation growth, mortality, or crippling effects only at 1.21 μg/L or greater, and reproductive effects at 0.63 μg/L, all of these concentrations being higher than mean levels for the continuously dosed stream (except for the

three days at 0.801 μg/L). AChE inhibition should be a reasonably good sublethal effect indicator because data from this study and Jarvinen et al [*12*] show that the response is rapid and remains stable over time at a given chlorpyrifos concentration. Percent inhibition at two continuous stream concentrations (24% at 0.14 μg/L and 41% at 0.42 μg/L) falls on or very near the concentration-effect curves obtained by Jarvinen et al [*12*] from the laboratory chronic test. From this relationship, it is reasonable to presume that other effects would be similar under equivalent exposure conditions in the laboratory and field. Jarvinen et al [*12*] observed slight but significant growth effects on second generation minnows at 0.12-μg chlorpyrifos/L, which inhibited AChE in parent fish by 10% or less. No equivalent laboratory data (repeated biweekly 24-h pulses) are available to which to compare the pulse stream results.

AChE activity was despressed further by pulsed chlorpyrifos exposure than by comparable (15 times less) continuous concentrations but activity recovered within a few days in the pulse stream to levels somewhat less than control activity. This type of AChE response pattern is similar to those for four fish species, including fathead minnows and bluegills, exposed to several organophosphorus pesticides by Weiss [*40*], and to that for mummichogs exposed to chlorpyrifos by Thirugnanam and Forgash [*41*]. The greater the pulse concentration, the greater the initial AChE reduction, and the lower the extent of recovery although the recovered level was consistently higher than comparable concentrations of continuous exposure levels of inhibition. This would indicate that the cumulative effects of brief but repeated higher levels of inhibition had a greater negative impact (crippling) than somewhat lower but continuous levels of inhibition. Such an effect contradicts the concept of the impact equivalency of numerically equal "exposure units" [*39*] derived by using substantially different exposure concentrations and durations. Another consideration is that in monitoring AChE activity levels as an indicator of pesticide exposure, higher (more normal) levels of activity might be observed in association with effect concentrations of chlorpyrifos than lower (more abnormal) levels associated with no-effect concentrations. Further information, most appropriately developed in the more controllable laboratory environment, is needed to fully understand these relationships.

Bluegills were also affected only in the pulse stream. The typical organophosphorus pesticide poisoning symptoms [*38*] of lethargy, tetanic spasms, and forward involuntary extension of the pectoral fins were observed in both free roaming and caged bluegills. Unlike fathead minnows, however, bluegills appeared to recover fully two to four days after the pulses and showed no signs of crippling or any other adverse effects. Eaton [*38*] observed that bluegills showing these symptoms during a laboratory continuous chronic exposure to malathion nearly always died and that crippling also occurred at these exposure concentrations. He also observed that reproduction and early development of second generation fish was not affected among those individuals that survived these concentrations.

In general, bluegill AChE results were similar to those for fathead minnows; peak concentrations in pulses caused greater inhibition (60%) than corresponding inhibition (40%) during continuous exposure to eleven times lower concentrations. Here again, it was the peak rather than the continuous average level that had the greater effect. Acute toxicity (96-h LC_{50}) data indicate that bluegills are approximately 100 times more sensitive to chlorpyrifos than fathead minnows [*11,13,34*], but their sensitivity to AChE inhibition resulting from continuous or 24-h pulse exposure appears very similar. It is also interesting that if the acute-chronic ratio for fathead minnows of 1000 [*12*] is applied to bluegill acute values (about 3 μg/L), a concentration of 0.003 μg/L would be projected as the threshold value for chronic toxicity. This value is 40 to 100 times lower than the continuous stream concentration, which caused no observable effects in this study. These relationships might indicate that for organophosphorus pesticides, the degree of AChE inhibition is a better way of estimating chronic toxicity from short exposures than application of the acute-chronic ratio for one species to the acute value for other species (application factor concept).

White suckers were the most sensitive of the three species studied. They died in the pulse stream and showed signs of being affected by continuous stream concentrations that reduced AChE activity by 59%. No other sources of information were found on the effects of chlorpyrifos on white suckers.

Chlorpyrifos was accumulated rapidly by fatheads and bluegills to steady state levels (within a day or two) and depurated at a rate of about 50% per day. Fathead minnows, which had five times higher lipid concentrations than bluegills at the end of the study, accumulated about five times as much chlorpyrifos. For reasons that are not understood, BCFs for continuously exposed fathead minnows in this study were about 1/3 of those obtained by Jarvinen et al [*12*] in their laboratory chronic test. BCFs for bluegills were also lower than for most literature values [*13,42*]. Data from the latter two references are from instantaneous applications to ponds, and both show an increase in BCFs over one to three weeks as water concentrations dropped. It is possible that the apparent increases in BCF from these studies is due not to increased accumulation but to a lag in depuration resulting in nonsteady state residue concentrations at the time water concentrations were measured. BCFs for two closely related species, pumpkinseed sunfish and rock bass [*43*], were 21 and 90 after seven days continuous exposure to 0.4-μg/L chlorpyrifos in an artificial stream, or much closer to our bluegill values. These data demonstrate that monitoring tissue residues frequently fails to provide insight into the extent of exposure because they do not adequately characterize rapidly changing exposure conditions in the environment.

A major reason for conducting this study in the artificial outdoor streams was to evaluate their utility for elucidating system level effects. For example, we hoped to be able to determine the influence of toxicant-related changes in macroinvertebrate populations on the ability of fish to obtain food. Production of fathead minnows and growth of bluegills indicate that the macroinver-

tebrate species shifts and reductions did not reduce food availability. Based on stomach contents, the bluegills appeared to be opportunistic in their feeding habits, eating whatever was most numerous or easily obtained. This is reflected in the large number of chironomid larvae in the May sampling when amphipods and isopods were more numerous but probably less obtainable in the continuous and control streams, and the periodic high number of cladocerans found in stomachs. Krska and Applegate [*44*] observed the same opportunistic feeding habits among bluegills in heated and unheated areas of a power plant cooling reservoir. The greater variety and balance of different species consumed by control fish in the later sampling periods is probably a reflection of the greater macroinvertebrate species diversity maintained in the control stream through the end of the test.

Effects of chlorpyrifos in this experiment on "community level" characteristics, such as stream production/respiration ratios, plant litter decomposition, nitrate concentration as an indicator of organic matter mineralization, total organic carbon production, and microbial biomass production and heterotrophic activity, are described by Pritchard [*28*]. All of these, with the possible exception of plant tissue decomposition, were unaffected in the chlorpyrifos-dosed streams as compared to the control stream. The sensitivity of several of the measurements, such as nitrate and total organic carbon concentrations, may have been masked by input from the Mississippi River water. Additional laboratory and on-site microcosm studies, however, showed that 10 to 100 times higher than the stream concentrations of chlorpyrifos were also generally nontoxic. The rate of decomposition of the submerged macrophyte *Potamogeton* (pondweed) appeared to be reduced numerically but not statistically in chlorpyrifos-dosed streams. The response was greater in the continuous than in the pulse stream, and was not observed in tests with the shore grass *Agrostis* (red top). In general, the results indicate that these "community level" indices are less sensitive than several other fish and invertebrate effect indicators, although macroinvertebrate diversity might be considered a community level measure and was affected. The state of these indices would, therefore, be poor indicators (except for macroinvertebrate diversity) of the health of aquatic ecosystems suspected or known to be contaminated by chlorpyrifos.

The nontraditional design of this experiment used neither a range of concentrations in separate streams nor replicates of any treatment condition, yet still provided reliable information about the effects of chlorpyrifos on aquatic ecosystems. This was achieved to a large extent because of the substantial amount of laboratory data obtained in connection with this study or already available. Another important factor was that data were available from previous Monticello stream studies, especially those from control or reference streams. Design compromises are often necessary in conducting resource consumptive field studies, and sometimes the vagaries of nature complicate application of the "rules" of toxicology as they are meant to be applied in the lab. For example, one of three streams in this experiment (the pulse stream)

was particularly different from the other two at the start of the test period, probably making it nonreplicable. Even dividing a single stream midway between the two ends and dosing the lower half would have drawbacks because the habitat and biota are different at each end. It has been suggested that having replicate control streams in future similar studies might provide a useful estimate of between-stream variability.

A final analysis of the effect relationships of the various system components is probably not possible because the study was not long enough. Early life stages of fish that reproduced were not exposed, responses of the macroinvertebrate community were incomplete, many seasonal influences were not investigated, and so on. It is likely that it takes an entire aquatic community longer to reach a reasonably steady state while being perturbed than it takes to produce and observe effects on the life-cycle responses of single species components in laboratory toxicity tests [*44*]. Thus, what was obtained from this study is probably only an aspect of what would ultimately occur (that is, only a fraction of the ecosystem's total capacity to perform under this perturbation [*45*]), and it is difficult to place the results into their proper perspective. This is not to say that useful information was not obtained. Indeed, from a very practical viewpoint, the duration of the chlorpyrifos insults to the streams was probably longer than many resulting from actual pest control applications, and, therefore, the results may be more "real" than might result from a much longer study. The relative chronic sensitivity of the stream ecosystems (that is, any form of effect) versus that of laboratory-testable individual species was also not resolved, assuming such an analysis is really feasible. Stream sensitivities of amphipods and fathead minnows seemed close to those in the laboratory, and they appeared so for other species as well (for example, bluegills and chironomids). One "higher level of organization" response, reduced species diversity, was observed in both streams but is essentially only a reflection of effects on individual species and, therefore, is not necessarily different (depending on the sensitivity of the laboratory tested species). No evidence of energy flow disruption to fishes was observed. Better resolution of the question would require longer experiments and probably the use of several constant toxicant concentrations. Sophisticated microcosm experiments might be a satisfactory alternative for some purposes. Further investigation of exposure duration and concentration relationships and intermittent exposure effects should mainly be through laboratory studies with individual species. Results from these studies should lead to development of effects predictive models that can be selectively and strategically field tested in appropriate outdoor systems.

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